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Direct Comparison of the BD Phoenix System with the MicroScan WalkAway System for Identification and Antimicrobial Susceptibility Testing of *Enterobacteriaceae* and Nonfermentative Gram-Negative Organisms▽

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The Phoenix automated microbiology system (BD Diagnostics, Sparks, MD) is designed for the rapid identification (ID) and antimicrobial susceptibility testing (AST) of clinically significant human bacterial pathogens. We evaluated the performance of the Phoenix instrument in comparison with that of the MicroScan WalkAway system (Dade Behring, West Sacramento, CA) in the ID and AST of gram-negative clinical strains and challenge isolates of *Enterobacteriaceae* ($n = 150$) and nonfermentative gram-negative bacilli (NFGNB; 45 clinical isolates and 8 challenge isolates). ID discrepancies were resolved with the API 20E and API 20NE conventional biochemical ID systems (bioMerieux, Durham, NC). The standard disk diffusion method was used to resolve discordant AST results. The overall percentages of agreement between the Phoenix ID results and the MicroScan results at the genus and species levels for clinical isolates of *Enterobacteriaceae* were 98.7 and 97.7%, respectively; following resolution with conventional biochemical testing, the accuracy of the Phoenix system was determined to be 100%. For NFGNB, the levels of agreement were 100 and 97.7%, respectively. Both systems incorrectly identified the majority of the uncommon nonfermentative nonpseudomonal challenge isolates recovered from cystic fibrosis patients; these isolates are not included in the databases of the respective systems. For AST of *Enterobacteriaceae*, the rate of complete agreement between the Phoenix results and the MicroScan results was 97%; the rates of very major, major, and minor errors were 0.3, 0.2, and 2.7%, respectively. For NFGNB, the rate of complete agreement between the Phoenix results and the MicroScan results was 89.1%; the rates of very major, major, and minor errors were 0, 0.5, and 7.7%, respectively. Following the confirmatory testing of nine clinical isolates initially screened by the MicroScan system as possible extended-spectrum-β-lactamase (ESBL)-producing organisms (seven *Klebsiella pneumoniae* isolates and two *Escherichia coli* isolates), complete agreement was achieved for eight isolates (one ESBL positive and seven negative); one false positive was obtained with the Phoenix instrument. The MicroScan system correctly detected the 10 ESBL challenge isolates, versus the 6 detected by the Phoenix system. Overall, there was good correlation between the Phoenix instrument and the MicroScan system for the ID and AST of *Enterobacteriaceae* and common NFGNB. The Phoenix system is a reliable method for the ID and AST of the majority of clinical strains encountered in the clinical microbiology laboratory. Until additional performance data are available, results for all *Klebsiella pneumoniae* or *Klebsiella oxytoca* and *E. coli* isolates screened and confirmed as ESBL producers by any automated system should be confirmed by alternate methods prior to the release of final results.

Clinical microbiology laboratories are under increasing pressure and scrutiny from clinicians and administrators to provide rapid, accurate, and timely bacterial identification (ID) and antimicrobial susceptibility testing (AST) results. The primary goals in providing such information are to aid clinicians in the diagnosis and treatment of infectious diseases, influence the appropriate selection of antimicrobial therapy, monitor community and institutional resistance patterns, and contribute to a reduction in health care-associated costs. To accomplish these goals, many laboratories have implemented the use of semiautomated microbial ID and AST instruments that have been designed to reduce turnaround times, increase efficiency, and improve cost-effectiveness (14). The Phoenix automated microbiology system (BD Diagnostic Systems, Sparks, MD) is the most recent instrument to receive clearance from the Food and Drug Administration (FDA) to provide rapid ID and AST of gram-negative and gram-positive bacteria recovered from human specimens.

In this study, we performed a side-by-side direct evaluation of the Phoenix instrument and our in-house automated system, the MicroScan WalkAway 96 system (Dade Behring, West Sacramento, CA). The objectives of the study were threefold: (i) to evaluate the performance and accuracy of the Phoenix instrument for the ID and AST, under daily routine laboratory operating conditions, of gram-negative clinical isolates from the family *Enterobacteriaceae* and nonfermentative gram-negative bacilli (NFGNB) that reflect our organism mix; (ii) to challenge the respective systems by testing a set of previously characterized

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NFGNB clinical isolates that are not listed in the databases of the respective systems; and (iii) to screen and detect extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* or *Klebsiella oxytoca* isolates, including a set of challenge ESBL isolates previously characterized and provided as unknowns by an independent laboratory.

(The findings of this study were presented in part at the 106th General Meeting of the American Society for Microbiology, Orlando, FL, 2006 [15].)

MATERIALS AND METHODS

Bacterial isolates. A total of 195 fresh clinical isolates (150 strains of *Enterobacteriaceae* and 45 strains of NFGNB) recovered from routine cultures in the University of Louisville Hospital clinical microbiology laboratory, Louisville, KY, were tested. To ensure that an appropriate mix of species was tested, the numbers of *E. coli* and *Pseudomonas aeruginosa* isolates were restricted to 30 for each organism. The following genera and species made up the bacterial mix: *E. coli* (25 isolates), *Klebsiella pneumoniae* (45 isolates), *Klebsiella oxytoca* (9 isolates), *Enterobacter cloacae* (25 isolates), *Enterobacter aerogenes* (11 isolates), *Serratia marcescens* (10 isolates), *Proteus mirabilis* (18 isolates), and *Proteus vulgaris*, *Morganella morganii*, *Salmonella* sp., *Citrobacter freundii*, *Pantoea* (*Enterobacter*) *agglomerans*, and *Yokenella* (*Koserella*) *regensburgei* (1 isolate each); representative NFGNB consisted of *Pseudomonas aeruginosa* (26 isolates), *Acinetobacter baumannii* (8 isolates), *Stenotrophomonas maltophilia* (8 isolates), and one isolate each of *Chromobacterium violaceum*, *Kingella* sp., and *Pseudomonas fluorescens* or *Pseudomonas putida*. A challenge set of eight previously well characterized NFGNB isolates with low frequencies of occurrence that had been recovered primarily from cystic fibrosis patients consisted of *Ralstonia mannitolilytica* (two isolates), *Burkholderia cenocepacia* (three isolates), and one isolate each of *Burkholderia multivorans*, *Burkholderia gladioli*, and *Pandoraea pnomenusa* (4). In addition to clinical isolates of *E. coli* and *Klebsiella pneumoniae*, a set of 10 ESBL-producing challenge isolates previously characterized by appropriate biochemical or molecular methods, including isoelectric focusing, substrate profiling, inhibitor profiling, plasmid isolation, recombinant DNA techniques, sequencing, and transformation, were tested by the respective automated systems to examine the abilities of the systems to detect ESBL production. These isolates were previously confirmed to harbor and produce β-lactamases known to cause false-positive and false-negative Clinical and Laboratory Standards Institute (CLSI) confirmatory test results, such as high-level AmpC, SHV-1, and K1 β-lactamases and class A carbapenemases, as well as multiple other β-lactamases (up to five enzymes) (11, 18).

All isolates were concurrently processed by the Phoenix and MicroScan systems for genus and species ID and the determination of the antimicrobial susceptibility category. Inoculum suspensions for the respective systems were processed on the same day using the same fresh 18- to 24-h-subculture plate. Separate purity plates were inoculated from each system's basic inoculum, and the resulting subcultures were used for discrepancy resolution (for discordant ID and AST results) and the assessment of culture purity.

Phoenix ID and AST. Phoenix NMIC/ID-108 combo panels (combined ID and AST cards) were inoculated and incubated according to the manufacturer's recommendations. BDExpert software versions 3.34A and 3.54A were used in this evaluation. The bacterial ID method employs modified conventional fluorogenic and chromogenic substrates in a system of 45 microwells containing dried biochemical substrates and 2 fluorescent control wells (2, 8). A suspension of each isolate, following adjustment to a 0.5 McFarland standard by using a CrystalSpec nephelometer (BD Diagnostics), was poured into the designated ID chamber of the Phoenix panel after a 25-μl aliquot was removed for AST. The AST section of the combination panel consists of 84 microwells containing dried antimicrobials and one growth control well. The method is a broth-based microdilution test that utilizes a redox indicator to enhance the detection of organism growth. As previously indicated, 25 μl of the standardized ID broth suspension was transferred into the AST broth, which resulted in final concentration of approximately 5×10^5 CFU/ml. The Phoenix ESBL test is based on the detection of growth in the presence of cefpodoxime, ceftazidime, ceftriaxone, and cefotaxime with or without clavulanic acid. The BDExpert system (versions 3.34A and 3.54A) consists of a series of rules, which are activated by MIC data, by the identity of the bacterial species, or by the result of the ESBL test. At the time of this study, only rule no. 1505, "isolate is confirmed positive for extended-spectrum beta-lactamase; consultation with an infectious disease practitioner is recommended," was incorporated into the program, but interpretative rules for *Citrobacter*, *Enterobacter*, *Morganella*, *Proteus*, and *Serratia* were not available in the United States as a result of the FDA's restriction of testing for ESBL production to *E. coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca.* All panels were sealed with a panel closure and loaded into the instrument. Quality control and maintenance were performed according to the manufacturer's recommendations. All organism preparations were logged and loaded into the instrument within the required 30-min timeline. Final results are available in 2 to 12 h for ID and 4 to 16 h for AST. AST results are expressed as an MIC with an accompanying category (susceptible, intermediate, or resistant) interpretation.

MicroScan ID and AST. The MicroScan WalkAway 96 system served as the primary reference method for ID and AST. ID and AST (using breakpoints with category interpretations) were performed with Neg BP combo panel type 30. All procedures were performed according to the manufacturer's recommendations. The screening of ESBL-producing microorganisms and interpretation were based on rules contained within the LabPro Expert system (version 2.0), which uses growth in the presence of cefpodoxime (4 μg/ml) and ceftazidime (1 μg/ml) at concentrations recommended by the CLSI for ESBL screening (3). The FDA limits screening for ESBLs by this system to *E. coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* isolates, as outlined in the CLSI guidelines (3). Other members of the family *Enterobacteriaceae*, such as *Citrobacter* spp., *Enterobacter* spp., *Serratia* spp., and members of the *Proteus* group (for which FDA approval and CLSI guidelines for testing were added in 2007), are known to harbor AmpC β-lactamase enzymes and may additionally produce ESBLs that give a positive ESBL screening result. However, the expert system does not support the detection of derepressed AmpC β-lactamases and ESBL production in these organisms and does not alert the user to the possibility of ESBL production (20), although organisms with this type of β-lactamase would test as resistant.

The CSLI four-disk diffusion confirmatory method (including cefotaxime and ceftazidime with and without clavulanic acid) was used for the confirmation of ESBL phenotypes of the relevant clinical and challenge isolates of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *E. coli* (3).

Discrepancy resolution. The ID results from the Phoenix and MicroScan systems were compared to determine the agreement at the genus and species levels. The ID was considered to be correct when the results from the two systems agreed at both levels. If the genus- and/or species-level IDs from the two systems were not in agreement, the isolate was retested in duplicate by both systems. If the discrepancy was not resolved, the organism was tested with the API 20E or API 20NE (internal arbitration). For the strains for which the Phoenix or MicroScan result was concordant with the third (internal-arbitration) result, this ID was considered to be correct. If the third result (from internal arbitration) did not agree with either of the original discrepant IDs, the isolate in question was excluded from the study.

Discrepant AST results were resolved by retesting the isolate in parallel with both systems, and the respective expert systems were applied for category concordance for the repeat results. If the AST results remained discrepant, the isolate was tested by the CLSI disk diffusion method (3). Rates of errors, categorized as very major errors (VMEs), major errors (MEs), and minor errors (mEs), were calculated for each system, but only VMEs and MEs were resolved.

RESULTS

Bacterial ID. Table 1 shows the results of testing of 150 isolates of *Enterobacteriaceae* and 45 NFGNB. The Phoenix and MicroScan systems showed percentages of agreement at the genus and species levels of 98.7 and 97.7%, respectively, for the various isolates of *Enterobacteriaceae*. Two isolates of the *Enterobacteriaceae* family required the resolution of results by testing with the API 20E. One isolate was initially identified by the MicroScan system as *Yokenella* (*Koserella*) *regensburgei* and as *Hafnia alvei* by the Phoenix system. The MicroScan system identified the second isolate as *Serratia fonticola* or *Enterobacter aerogenes*, versus the Phoenix ID as *Enterobacter aerogenes* or *Enterobacter cloacae*. Following confirmatory testing, the Phoenix system was found to have correctly identified both isolates with discrepant results, which gave an overall accuracy of the Phoenix system of 100% for the ID of *Enterobacteriaceae* to the genus and species levels.

For the 45 NFGNB isolates, two discrepant IDs by the Phoenix system were noted. One isolate, a mucoid strain of

TABLE 1. ID results for *Enterobacteriaceae* and nonfermenters

Organism	No. of isolates	% Of isolates for which Phoenix ID was:		API 20E ID
		Concordant with MicroScan ID	Discordant with MicroScan ID	
Enterobacteriaceae				
Klebsiella pneumoniae	45	100	0	
E. coli	25	100	0	
Enterobacter cloacae	25	100	0	
Proteus mirabilis	18	100	0	
Enterobacter aerogenes	11	100	0	
Serratia marcescens	10	100	0	
Klebsiella oxytoca	9	100	0	
Proteus vulgaris	1	100	0	
Morganella morganii	1	100	0	
Salmonella sp.	1	100	0	
Citrobacter freundii	1	100	0	
Pantoea (*Enterobacter*) *agglomerans*	1	100	0	
Yokenella (*Koserella*) *regensburgei*	1	0	100	*Hafnia alvei*[a]
Serratia fonticola or *Enterobacter aerogenes*	1	0	100	*Enterobacter aerogenes*[b]
Total	150			
Nonfermenters				
Pseudomonas aeruginosa	26	96.2	3.8	Mucoid *Pseudomonas aeruginosa*[c]
Acinetobacter baumannii	8	100	0	
Stenotrophomonas maltophilia	8	100	0	
Chromobacterium violaceum	1	100	0	
Moraxella sp.	1	0	100	*Kingella kingae*[d]
Pseudomonas fluorescens or *Pseudomonas putida*	1	100	0	
Total	45			

[a] Phoenix ID: *Hafnia alvei*.
[b] Phoenix ID: *Enterobacter aerogenes* or *Enterobacter cloacae*.
[c] Phoenix ID: *Pseudomonas putida* or *Pseudomonas aeruginosa* (*Pseudomonas fluorescens* or *Pseudomonas putida* upon repeat testing).
[d] Phoenix ID: *Kingella kingae*.

Pseudomonas aeruginosa, was misidentified as *Pseudomonas putida* or *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* or *Pseudomonas putida.* Following confirmatory testing, the isolate was identified as *Pseudomonas aeruginosa.* The second discrepant isolate, following confirmatory testing, was found to have been correctly identified by the Phoenix system as *Kingella kingae*, versus an incorrect ID by the MicroScan system as *Moraxella* sp. The overall accuracy of the Phoenix system for the ID of NFGNB was 100% at the genus level and 97.7% at the species level.

Each system had difficulty in providing precise IDs of the eight challenge NFGNB isolates (Table 2). Both systems correctly identified the two strains of *Ralstonia mannitolilytica* to the genus level but misidentified both strains as *Ralstonia pickettii.* Of the five *Burkholderia* isolates, the MicroScan and Phoenix systems correctly identified one and three isolates, respectively, to the genus level; all five isolates were misidentified at the species level by both systems. The Phoenix system yielded a "no ID" message for the strains of *Burkholderia multivorans* and *Burkholderia cenocepacia*, whereas the MicroScan system generated a "no ID" result for two strains of *Burkholderia cenocepacia. Pandoraea pnomenusa* was incorrectly identified as *Alcaligenes xylosoxidans* and *Moraxella* sp. by the MicroScan and Phoenix systems, respectively. *Burkholderia gladioli* was misidentified by the MicroScan system as *Acinetobacter baumannii*, while the Phoenix system identified the isolate as *Burkholderia* sp. or *Ralstonia* sp.

Antimicrobial susceptibility. Antimicrobial susceptibility results for the *Enterobacteriaceae* and NFGNB isolates tested against individual antibiotics are summarized in Table 3. Category agreement (CA) was defined as matching classifications as susceptible, intermediate, or resistant by the two systems. The level of CA for the *Enterobacteriaceae* was 96.5%. The mE,

TABLE 2. Summary of ID results for challenge isolates

MicroScan ID (% probability)	Phoenix ID	Reference ID
Ralstonia pickettii	*Ralstonia pickettii*	*Ralstonia mannitolilytica*
Ralstonia pickettii (51) or *Burkholderia cepacia* (42)	*Burkholderia cepacia* or *Ralstonia pickettii*	*Ralstonia mannitolilytica*
Burkholderia cepacia	No ID	*Burkholderia multivorans*
Alcaligenes xylosoxidans	*Moraxella* sp.	*Pandoraea pnomenusa*
No ID	*Burkholderia cepacia*	*Burkholderia cenocepacia*
Vibrio sp. or *Chryseobacterium* sp.	*Burkholderia cepacia*	*Burkholderia cenocepacia*
No ID	No ID	*Burkholderia cenocepacia*
Acinetobacter baumannii	*Burkholderia* sp. or *Ralstonia* sp.	*Burkholderia gladioli*

TABLE 3. AST results for *Enterobacteriaceae* and gram-negative nonfermenters

Organism group and antibiotic	Total no. of isolates tested	% CA	No. of isolates categorized as[a]:			No. (%) of isolates associated with:		
			S	I	R	mE	ME	VME
Enterobacteriaceae								
Amikacin	132	100.0	132	0	0	0	0	0
Gentamicin	131	98.0	124	4	3	3 (2.3)	0	0
Tobramycin	135	96.0	124	7	3	4 (2.9)	0	0
Imipenem	119	100.0	119	0	0	0	0	0
Meropenem	131	100.0	131	0	0	0	0	0
Cephalothin	83	83.8	65	2	16	7 (8.4)	0	1 (6.2)
Ceftazidime	132	95.9	105	3	24	2 (1.5)	1 (0.9)	2 (8.3)
Cefotaxime	133	96.6	109	7	17	3 (2.3)	0	1 (5.8)
Cefepime	114	96.9	110	1	1	2 (1.7)	0	1 (33.3)
Aztreonam	132	94.6	114	2	2	3 (2.3)	3 (2.6)	2 (1.4)
Piperacillin	114	94.6	77	11	11	7 (6.1)	0	0
Amoxicillin-clavulanic acid	123	90.6	73	8	8	8 (6.5)	0	0
Trimethoprim-sulfamethoxazole	124	99.3	108	0	0	0	0	1 (6.2)
Ciprofloxacin	99	98.3	89	1	1	2 (2.0)	0	0
Gatifloxacin	132	98.0	121	1	1	2 (1.5)	1 (0.8)	0
Levofloxacin	132	98.6	121	0	0	2 (1.5)	0	0
Tetracycline	75	98.9	67	1	1	1 (1.3)	0	0
Total	2,041	96.5	1,789	48	48	46 (2.4)	5 (1.4)	8 (6.0)
Nonfermenters								
Amikacin	35	91.4	31	4	0	3 (8.6)	0	0
Gentamicin	35	82.9	26	5	4	5 (14.2)	1 (2.9)	0
Tobramycin	35	94.3	32	1	2	2 (5.7)	0	0
Imipenem	35	100.0	32	0	4	0	0	0
Meropenem	35	100.0	32	0	3	0	0	0
Ceftazidime	35	97.1	32	1	2	1 (2.9)	0	0
Cefepime	26	84.6	19	6	1	4 (15.4)	0	0
Piperacillin	35	94.3	29	2	4	2 (5.7)	0	0
Ciprofloxacin	35	97.1	25	0	10	1 (2.9)	0	0
Levofloxacin	42	90.5	30	2	10	4 (9.5)	0	0
Total	348	93.2	288	21	40	22 (8.0)	1 (2.9)	0

[a] S, susceptible; I, intermediate; R, resistant.

ME, and VME rates were 2.4, 1.4, and 6.0%, respectively. The majority of VMEs were associated with β-lactam antibiotics. β-Lactam antibiotics also accounted for the majority of MEs. Based on the indications of the MicroScan ESBL screening software (version 2.0), nine clinical isolates (seven *Klebsiella pneumoniae* and two *E. coli* isolates) were further screened as possible ESBL producers (Table 4). Following confirmatory testing, six *Klebsiella pneumoniae* isolates were determined to be negative for ESBL production and one was found to be positive; both *E. coli* isolates were negative. The Phoenix system correctly detected the one ESBL-producing *Klebsiella pneumoniae* isolate and incorrectly detected one of the two *E. coli* isolates as an ESBL-producing organism (Table 4). Of the 10 previously characterized ESBL-producing challenge isolates (5 *Klebsiella pneumoniae*, 3 *Klebsiella oxytoca*, and 2 *E. coli* isolates), the Phoenix instrument correctly identified 6 isolates, with a "no alert" message recorded for 4 isolates; the MicroScan system correctly classified all 10 isolates as possible ESBL producers with the message "?ESBL," indicating the need for confirmatory testing.

Overall complete agreement between the two systems following AST of NFGNB was 93.2%; the mE, ME, and VME rates were 8.0, 2.9, and 0%, respectively. Gentamicin and trimethoprim/sulfamethoxazole accounted for the two MEs.

DISCUSSION

Overall, the Phoenix instrument compared favorably to the MicroScan system for the ID and susceptibility testing of gram-negative bacilli. We purposely restricted the number of *E. coli* and *Pseudomonas aeruginosa* isolates to ensure a balanced representation of clinical isolates, especially those with low frequencies of occurrence in our laboratory. Although the range and number of isolates tested were not as broad as those described in comparable published reports, they do reflect a realistic representation of the organisms encountered by a service-oriented clinical microbiology laboratory. The overall agreement for the ID of common members of the family *Enterobacteriaceae* was similar to that reported by others, although in the previously reported studies, the performance of the Phoenix instrument was compared directly to conventional ID systems (1, 2, 12). In contrast, O'Hara tested a set of challenge isolates consisting of biochemically typical and atypical stock culture representatives of the *Enterobacteriaceae* and

TABLE 4. Detection of ESBL-producing challenge and clinical isolates

Organism	Result[a] from:		Disk confirmation	ESBL	Other β-lactamase(s)
	MicroScan	Phoenix			
Clinical strains					
Klebsiella pneumoniae	?ESBL	No alert	ND		
	?ESBL	No alert	ND		
	?ESBL	No alert	Negative		
	?ESBL	No alert	Negative		
	?ESBL	No alert	Negative		
	?ESBL	No alert	Negative		
	?ESBL	1505	Positive		
E. coli	?ESBL	1505	Negative		
	?ESBL	No alert	Negative		
Challenge strains					
Klebsiella pneumoniae	?ESBL	1505		SHV-3-like ESBL	SHV-1-like β-lactamase
	?ESBL	1505		CTX-M-19	SHV-1-like and TEM-1-like β-lactamases
	?ESBL	No alert		SHV-4-like ESBL	PSE-like, FOX-like AmpC, and SHV-1-like β-lactamases
	?ESBL	No alert		SHV-5 and SHV-3	ACT-1 AmpC, SHV-1, and TEM-1
	?ESBL	No alert		SHV-3-like ESBL	SHV-1-like β-lactamase
Klebsiella oxytoca	?ESBL	No alert		SHV-3-like ESBL	DHA-like AmpC, K1, and TEM-1-like β-lactamases
	?ESBL	1505		SHV-1-like ESBL	K1 and TEM-1-like β-lactamases
	?ESBL	1505		TEM-3-like ESBL	K1
E. coli	?ESBL	1505		TEM-12	TEM-1
	?ESBL	1505		TEM-10	
Serratia marcescens	?ESBL[1]	No alert		SHV-4-like β-lactamase	AmpC (chromosomal)
Enterobacter cloacae	?ESBL[1]	No alert		SHV-5-like β-lactamase	AmpC (chromosomal) and TEM-1
Citrobacter koseri	?ESBL[1]	No alert		SHV-7	TEM-1-like and OXA-like β-lactamases
Proteus mirabilis	?ESBL[1]	No alert		CTX-M-2	TEM-1-like β-lactamase

[a] ?ESBL, positive ESBL screen as defined by software using CLSI guidelines (no confirmation available on panel); ?ESBL[1], positive ESBL screen as defined by user (no confirmation available on panel); 1505, Phoenix system alert for a confirmed ESBL producer; ND, not done.

reported that the Phoenix system correctly identified only 89.9% to the genus and species levels (13). The majority of errors reported in her study were associated with the genus *Salmonella*, but no such errors were encountered in the present study. In the study by Carroll et al. (2), an *Enterobacter* (*Pantoea*) species, specifically *Pantoea agglomerans*, was misidentified as *Shigella flexneri*, one isolate of *E. coli* was misidentified as *Salmonella* sp., and all *Shigella* isolates were misidentified as *E. coli.* In our study, only two isolates required the resolution of results with the API 20E, and in these cases, the MicroScan system misidentified *Hafnia alvei* as *Yokenella* (*Koserella*) *regensburgei* and *Enterobacter aerogenes* as *Serratia fonticola* or *Enterobacter aerogenes.* Following the resolution of the two discrepancies, the Phoenix instrument was found to have achieved an overall accuracy of ID of *Enterobacteriaceae* at the genus and species levels of 100%. However, it is important for microbiologists to be aware of the shortcomings of both automated and nonautomated ID systems, especially for isolates identified as *Shigella* or *Salmonella* spp., which require confirmatory ID by additional testing (agglutination or the detection of characteristic phenotypic features following growth on MacConkey agar), in addition to knowledge of the source of the specimen.

Following the resolution of two discrepancies, the Phoenix system was found to have accurately identified all of the NFGNB clinical isolates, including the *Kingella kingae* isolate, which was misidentified as *Moraxella* sp. by the MicroScan system, and the mucoid strain of *Pseudomonas aeruginosa*, even though *Pseudomonas putida* was part of the selection. The results for the ID of this group of organisms are similar to those reported by Endimiani et al. (7), Menozzi et al. (10), and Stefaniuk et al. (16). In contrast, O'Hara reported that of 57 isolates of NFGNB, 48 (84.2%) were correctly identified by the Phoenix system to the genus and species levels (12).

Both systems encountered considerable difficulty in identifying unusual isolates that are occasionally recovered, albeit in low frequencies, from cystic fibrosis patients, including *Burkholderia* species other than *Burkholderia cepacia* and *Ralstonia* and *Pandoraea* spp. Given that these organisms are rare and that *Pseudomonas aeruginosa* is the most common bacterium isolated from cystic fibrosis patients, these results are not surprising since these organisms are not included within the databases of the respective instruments. In fairness to both systems, *Burkholderia cenocepacia* is part of the *Burkholderia cepacia* complex and the organisms of this complex are difficult to distinguish biochemically. Other investigators have reported similar observations and concerns in utilizing automated systems for the ID of such organisms and have emphasized the importance of confirmatory testing of these traditionally difficult-to-identify organisms (1, 7, 12, 19). Our data also illustrate the potential problems that may be encountered when using these systems alone for the ID of unusual NFGNB and the need to perform confirmatory testing using more reliable phenotypic or genotypic methods.

In our study comparing AST results from the MicroScan and Phoenix systems, the CAs for both groups of organisms were

excellent. A significant weakness of this study was the lack of sufficient numbers of resistant isolates to accurately determine the true rates of MEs and VMEs. In a service-oriented clinical microbiology laboratory in which clinical isolates are recovered from clinical specimens, like the one in which this study was conducted, some types of bacterial resistance occur at very low frequencies, which compromises the ability to generate data that are essential for calculating accurate error rates. This problem is reflected in the VME rates that exceed the accepted standard of ≤3% (6). Of particular note was the observation that the majority of VMEs involved β-lactam antibiotics. Menozzi et al. reported similar observations and noted, as was observed in our study, nearly equal numbers of errors for the various organisms tested (10). Similar findings were also reported by Carroll et al. for the *Enterobacteriaceae* (2).

Currently, five studies have extensively evaluated the ability of the Phoenix system to detect ESBLs (2, 10, 16, 17, 20). In the most recent study, Wiegand et al. (20) performed a side-by-side comparison of the MicroScan, Vitek 2, and Phoenix systems by testing 150 putative ESBL producers that were blindly distributed to three participating laboratories following the biochemical and molecular characterization of β-lactamases, which served as the reference method. A total of 85 isolates were identified as ESBL producers by the reference method. The Phoenix system demonstrated the highest sensitivity (99%) for the detection of ESBLs, followed by the Vitek 2 (86%) and the MicroScan (84%) systems. Carroll et al. demonstrated that the Phoenix expert rules system correctly detected the six ESBL producers, three *E. coli* and three *Klebsiella* spp. isolates, encountered in their study (2). Sturenburg et al. (17) also reported a high degree of sensitivity (100%) for the Phoenix system following the testing of 34 ESBL-producing challenge strains. Similar observations were reported by Sanguinetti et al. (14), who determined the Phoenix system to have 100% sensitivity and 98.9% specificity for the detection of ESBLs in 11 species of *Enterobacteriaceae* that had been characterized previously by phenotypic and genotypic methods. In a study of 74 ESBL-producing clinical isolates of *E. coli* and *Klebsiella pneumoniae* and 17 genotypically characterized control strains, Leverstein-van Hall et al. (9) compared the Phoenix system to the Vitek 1 and Vitek 2 systems, with the accuracies of the three systems for detecting ESBLs reported to be 89, 78, and 83%, respectively. In our study, both systems performed very well in screening for and/or detecting ESBL production in clinical isolates of *Klebsiella pneumoniae* and *E. coli.* However, the MicroScan system demonstrated higher levels of sensitivity and specificity in screening the ESBL-producing challenge isolates of *E. coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* that contained other β-lactamases. The higher-level performance of the Phoenix system reported by Wiegand et al. (20) and Sanguinetti et al. (14) was most likely due to the availability in Europe of expanded expert rules software (Phoenix software version 4.05W and MicroScan software version 1.12) that included organisms other than *E. coli* and *Klebsiella pneumoniae*, which is not available in the United States and is awaiting FDA clearance and CLSI recommendations for the testing of other members of the *Enterobacteriaceae* family, with the exception of the recent addition of guidelines for *Proteus mirabilis.*

Overall, the Phoenix system compared favorably with the MicroScan WalkAway system and is an acceptable alternative for the ID and susceptibility testing of commonly encountered species of *Enterobacteriaceae* and non-glucose-fermenting gram-negative bacilli. However, caution should be used in relying on either system for use in the ID and susceptibility testing of less commonly encountered clinical isolates, such as those that are occasionally recovered from patients with cystic fibrosis.

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